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Noncoordinate Control of RNA, Lipopolysaccharide, and Phospholipid Syntheses During Amino Acid Starvation in Stringent and Relaxed Strains of *Escherichia coli*

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The syntheses of RNA, lipopolysaccharides, and phospholipids were measured simultaneously in stringent and relaxed cells of *Escherichia coli* during normal growth or starvation for amino acids. The synthesis of all these molecules was inhibited by amino acid starvation, but the reduction in synthesis was not coordinated.

During amino acid starvation of *rel*$^+$ strains of *Escherichia coli*, the synthesis of three classes of macromolecules is known to be reduced; these are RNA (5, 14, 17), phospholipids (PL; 8, 15, 18), and the lipid A moiety of lipopolysaccharides (LPS; 19). Among the species of RNA, syntheses of rRNA and tRNA are more severely affected than the synthesis of mRNA (11, 13).

Polyacrylamide gel electrophoresis of lysates of radioactively labeled cells allows measurement of these components simultaneously in unfractionated cells (3). Using this technology, we investigated whether or not the syntheses of these three classes of macromolecules were coordinated during starvation for amino acids. Virtually all previous studies concerning amino acid starvation have measured only one macromolecule, and in the one study (15) in which both PL and RNA were followed, the species of RNA were not separately measured. The results described here are in general agreement with previous studies in the field, but emphasize that the synthesis of many macromolecules is reduced in amino acid-starved stringent cells and that the reduction is noncoordinated.

Strain CP78, *rel*$^+$ *arg his leu thr thi*, and the isogenic *rel* strain CP79 (6) were grown in a low-phosphate tris(hydroxymethyl)aminomethane-buffered medium (10) and starved for isoleucine, by adding valine (16), or for arginine, histidine, leucine, and threonine, by removing each of these amino acids separately from the medium. After starvation periods from 5 to 60 min, cultures were labeled with $^{32}P_i$ for 10 to 30 min. The results of these experiments were similar, and a representative one is shown in Table 1. The data given are for distribution of ^{32}P in the different bands. The total incorporation varied depending on the *rel* allele and treatment. The incorporation of $^{32}P_i$ by the starved stringent strain was seven times less than incorporation by the corresponding unstarved culture (see footnote *a*, Table 1). The distribution of the radioisotope in the starved stringent strain indicates a drastic relative decrease in rRNA and tRNA synthesis, whereas there is little change in the relative extent of mRNA and LPS labeling. In the same sample, labeling of PL was relatively increased. In the relaxed strain none of the measured macromolecules was drastically affected by starvation, but during all amino acid starvations the level of LPS labeling was consistently decreased by about 50%.

Since it is well known that chloramphenicol (CP) negates the stringent effect on RNA and PL syntheses during starvation (2, 8), parallel cultures were tested with CP. The results (Table 1) confirm this, but also show that CP inhibits the synthesis of LPS in either relaxed or stringent, starved or unstarved, cells.

To ascertain the amount of RNA in the ^{32}P-labeled material we treated some lysates from starved and unstarved cells with NaOH or with pancreatic ribonuclease and displayed them on gels (Fig. 1). Treatment with ribonuclease reveals that non-RNA material accumulated around the interface of the 5 to 13.5% polyacrylamide gel. In unstarved cells this material represents 1 to 2% of the total ^{32}P-labeled macromolecules, whereas in *rel*$^+$ starved cells the amount of this material can vary, and in some experiments it contained up to 20% of the total ^{32}P-labeled material. Some of this material is NaOH sensitive (Fig. 1). NaOH treatment hydrolyzes PL and increases the rate of migration of LPS (Fig. 1).

The experiments presented here show the usefulness of electrophoresis of the total cellular

TABLE 1. *Distribution of $^{32}P_i$ in macromolecules during amino acid starvation and treatment with CP*[a]

Treatment	^{32}P distribution (%) in:						
	rRNA	mRNA	5S + 6S	tRNA	LPS	PL	Other[b]
rel$^+$							
Control	31.2	10.1	2.0	8.1	10.8	23.7	14.1
−Leu	4.0	10.0	0.3	1.3	11.2	56.1	19.7
−Leu, +CP	21.2	17.2	1.0	6.5	3.9	21.6	28.6
+CP	24.3	25.7	1.3	8.2	5.4	24.8	10.3
rel							
Control	31.4	18.2	1.5	7.1	8.8	24.8	8.2
−Leu	27.0	24.5	1.6	6.9	3.8	21.1	15.1
−Leu, +CP	21.3	21.2	1.2	8.1	5.8	29.3	13.1
+CP	24.9	25.9	1.3	8.2	3.8	25.3	10.6

[a] Cultures (1 ml) of CP78 (*rel*$^+$) and CP79 (*rel*) were labeled for 30 min with 80 μCi of $^{32}P_i$ per ml in tris(hydroxymethyl)aminomethane medium supplemented with bases and requirements at 30°C. Starvation for leucine was achieved by suspending cells in medium devoid of leucine and carried out for 25 min before labeling. CP (200 μg/ml) was added 10 min before labeling. When CP was added to starved cells, cultures were starved for 25 min, and CP was added, followed 10 min later by $^{32}P_i$ addition. Whereas all lanes from the *rel* strain contained about the same number of cells, the material in the *rel*$^+$ starved lane contained about seven times as many cells as the control and CP lanes due to low incorporation of $^{32}P_i$ in the stringent starved cells. One hundred percent values represent 1.0×10^5 to 1.8×10^5 cpm. Labeling was stopped by placing cells on ice. Cells were centrifuged at 3,000 × *g* for 10 min at 4°C, washed once with dilution buffer (1), and centrifuged for another 10 min. After centrifugation the supernatants were poured off and the tubes were briefly drained. The cells were finally suspended in 100 μl of lysis buffer (7). The cells were heated for 2 min in boiling water and stored at −20°C overnight. Cell lysates (5 to 20 μl) were applied to two gels (3, 4, 7, 12). The first, a 5 to 13.5% tandem polyacrylamide slab gel, was used to separate the LPS, PL, and low-molecular-weight RNAs. The second, a 3% polyacrylamide gel, separated the 16S and 23S RNA and the DNA, which remains at the origin. The quantitation procedures were done according to Geggenheimer et al. and Kaplan and Apirion (7, 10). The quantitation of 16S and 23S rRNA and DNA was carried out on a 3% gel (4, 10), whereas quantitation of the mRNA fraction was computed by using data obtained from both gels. On a 5 to 13.5% polyacrylamide slab gel, the 16S rRNA and mRNA do not appear as distinct bands. They migrate to the interface between the 5% and the 13.5% portions of the gel, whereas the 23S rRNA and DNA remain at the origin. The interface of such a gel also contains some material that is not RNA since it is not hydrolyzed by ribonuclease (Fig. 1). This material is probably neither LPS nor PL. To determine the amount of mRNA in each sample, this material and the 16S RNA (as determined from the 3% gel) were subtracted from the material that migrated to the interface of the gel. We quantitated only lanes which contained at least 100,000 cpm; even so, the determination of components containing less than 3% of the total counts is not always reliable since the background, due mainly to smear, could be as much as one-third of that level.

[b] Such as the non-RNA material described in the text, DNA, material between tRNA and LPS, and smeared material between bands.

content in thin polyacrylamide slab gels. This technique can be applied to many different problems with a variety of organisms, provided that the cells can be lysed by the technique described here (or a similar one) and that the macromolecules being investigated can be distinctly identified in the gel.

The measurement of various RNA components, especially rRNA and mRNA, is reliably accomplished by this technique. Since rRNA is determined from a position on a gel, nascent rRNA chains will be missed, but since we labeled cells for relatively long periods (30 min), the fraction of nascent rRNA is small. Because the size of the mRNA molecules is heterogeneous they do not tend to migrate to a particular position in the gel, as is the case with rRNA, but on a 5 to 13.5% gel, the bulk of the mRNA is trapped at the interface between the two portions of the gel, since it is too large to enter the 13.5% portion of the gel but small enough to migrate to the bottom of the 5% portion. Also, during amino acid starvation of a *rel*$^+$ strain, the synthesis of rRNA is preferentially depressed, and therefore the contribution of 16S RNA to the RNA found in the interface of the gel is rather small (Fig. 1). Furthermore, our results are similar to those obtained by RNA-DNA hybridization techniques (see, e.g., reference 11), and varying labeling time from 10 to 30 min gives substantially similar results, further suggesting that the results reported here primarily reflect the synthetic capacity of these macromolecules. This conclusion is further supported

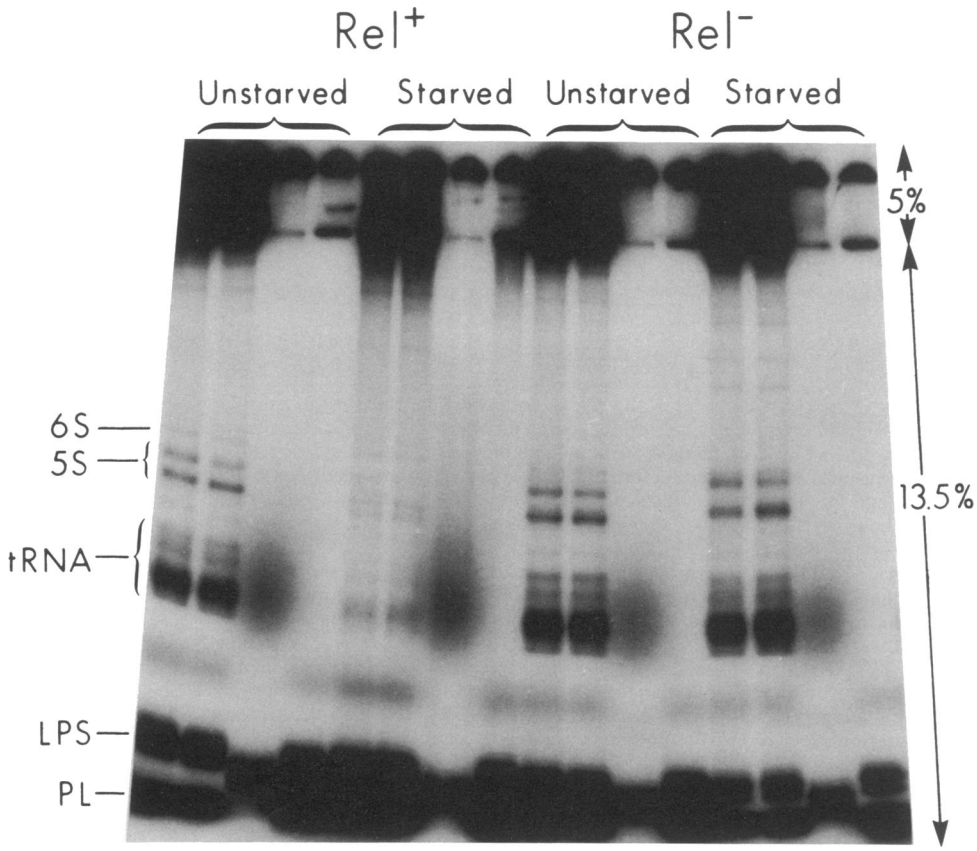

FIG. 1. *Presentation in a polyacrylamide gel of $^{32}P_i$-labeled macromolecules from stringent and relaxed strains. Four 2-ml samples (two of each strain) were grown in a low-phosphate tris(hydroxymethyl)-aminomethane-buffered medium (10) supplemented with bases (9) and requirements at 30°C and labeled with 50 μCi of $^{32}P_i$ per ml for 30 min. One sample from each strain was starved for isoleucine by adding valine for 20 min before labeling. The lysate of each sample was suspended in 0.4 ml of lysis buffer, which was then divided into four aliquots. One of these aliquots was untreated; the second was incubated and agitated in a 37°C water bath for 2 h; the third was agitated with NaOH (0.5 M, final concentration) at 37°C for 2 h, and the fourth was agitated with pancreatic ribonuclease (500 μg/ml, final concentration) at 37°C for 2 h. Following these procedures, the NaOH-treated samples were neutralized with HCl, and all the samples were applied to a 5 to 13.5% tandem gel (3). Material from aliquots one to four of each of the four samples appear from left to right.*

by the fact that the ^{32}P enters all these molecules from a common ATP pool.

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